BIOLOGICALS RESPONSE MODIFIERS ADVISORY COMMITTEE
Meeting #31
Briefing Document for October 25-26, 2001
Lentivirus Vectors in Gene Transfer Clinical Trials

Introduction

The first patient who participated in a gene therapy clinical trial in 1990 was given cells that had been exposed to a viral vector. The vector was derived from a murine gammaretrovirus, a member of the Retroviridae family. Since that time, gammaretrovirus-derived vectors continue to be tested in clinical trials when long-term gene expression is desired. Gammaretrovirus vectors integrate into the host genome, potentially resulting in long-term expression of the therapeutic gene for the life of the transduced cells and their progeny. However, one limitation to the use of gammaretroviral vectors is that they do not transduce non-dividing cells, such as macrophages, resting T lymphocytes, unstimulated hematopoietic stem cells, or neurons.

Gene transfer vectors based on lentiviruses are under development. They are being intensively studied because they provide the important advantage of being able to integrate and express genes in both dividing and non-dividing cells, hence broadening the potential pool of target cells for gene transfer. Notable *in vivo* animal studies include successful transduction of hematopoietic cells (18, 21), neurons (4, 16, 38), retinal cells (31), and the liver (27). There have also been successful *in vitro* transductions of other non-dividing cell types, including resting lymphocytes (6, 8), macrophages (24, 30), dendritic cells (7, 30), myocytes (14), and islet cells (11). Lentiviruses that have been developed as gene transfer vectors include human immunodeficiency virus (HIV) (24), simian immunodeficiency virus (SIV) (17), feline immunodeficiency virus (FIV) (29), and equine infectious anemia virus (EIAV) (19). HIV-based vectors have undergone the most development and are the focus of this document.

I. Issues Related to Manufacture of Lentivirus Vectors

This portion of the briefing package provides an overview of the current practices for manufacture of lentivirus-based vectors as well as discussion of safety issues associated with lentivirus-based vectors that could be addressed during manufacture (i.e., vector design) and quality control testing of the vector prior to clinical use. A primary safety concern associated with lentivirus-based vectors is the potential for recombination events that may generate a replication-competent lentivirus (RCL). This is of particular concern when the vector is based on a known human pathogen such as HIV.

A. Retroviral recombination

There are documented cases that describe generation of replication-competent retrovirus (RCR) during the course of manufacturing of gammaretrovirus-based vectors. For example, in a study by Otto and coworkers, molecular analysis of the RCR isolated from a production lot demonstrated that as little as 10 base pairs of nucleotide identity between packaging and vector sequences were sufficient to allow for recombination resulting in RCR generation (25). Mechanisms of genetic recombination are well

studied among gammaretroviruses. Homologous recombination can occur when two different RNA's are packaged into one virion. This recombination is a result of reverse transcriptase (RT) template switching (strand transfer) (13, 32). RT-mediated recombination has been documented to occur with HIV RT as well (26, 33). Additionally, recombination of HIV in HIV infected cells has been shown *in vitro* to alter the phenotype with respect to resistance to antiviral drugs (23). When macaques vaccinated with a live attenuated SIV were subsequently challenged with wild type SIV, a more virulent recombinant virus emerged that caused faster disease progression compared with non-vaccinated macaques (12).

Gammaretroviral vectors have been designed to minimize recombination between helper and vector sequences. Homologous recombination of retroviruses occurs at a frequency of 4×10^{-5} per base pair per replication cycle, while the rate of non-homologous recombination was measured to occur at a rate approximately 100-1000-fold lower than homologous recombination (36). Therefore, reduction in the amount of homology between vector and helper sequences will lower the likelihood of recombination events occurring. Additionally, many gammaretroviral vector packaging cell lines are designed with the helper sequences separated onto more than one plasmid (*i.e.*, separation of *env* and *gag-pol*). Dividing helper functions into two plasmids would require a minimum of two recombination events to generate an RCR, thus reducing the risk of generating an RCR even further. These essential safety features apply to lentiviral vectors as well.

B. Vector Mobilization

An additional concern associated with the use of lentiviral vectors in HIV-positive subjects is vector mobilization. Vector mobilization occurs when the vector genome is packaged by a wild-type HIV present in the same cell, by the same mechanisms that allow helper sequences to package vector genomes in the vector production system. Previous in vitro studies have shown that coinfection of cells with an HIV-based vector and wild-type HIV can result in effective mobilization of the vector sequences to additional cells (10). Vector mobilization in vivo beyond the intended target tissue, depending upon the nature of the transgene, may have safety consequences. However, mobilization of a vector designed to inhibit or prevent HIV replication or pathogenesis has been argued to have the potential to potentially enhance the therapeutic effect (10).

C. Elements of Lentivirus Vector Manufacturing and Design

Compared with gammaretroviruses, lentiviruses have a complex genome. As with all retroviruses, the genomes of lentiviruses contain the genes for *gag*, *pol*, and *env*. These genes code for the core structural proteins, viral enzymes, and envelope glycoproteins, respectively. HIV also contains six additional open reading frames for proteins involved in regulation of gene expression or pathogenesis. Tat and Rev are regulatory proteins that promote viral expression through transcriptional and postranscriptional mechanisms, respectively. There are also four accessory proteins, Vif, Vpr, Vpu, and Nef, that are involved in HIV replication and perhaps pathogenesis.

Developments in lentiviral vector manufacture and design benefited from lessons learned with gammaretroviral vector recombination. Specifically, features such as

limiting homology between vector and helper sequencing, and expressing *env* from a separate plasmid than *gag-pol* are used.

The 1st generation lentivirus vectors were produced via transient transfection in 293T cells using three plasmids (24):

1. The packaging plasmid contains all HIV viral genes, including accessory genes, with the exception of *env*. This plasmid carries the *trans*-acting helper sequences required to make a retroviral particle.
2. The envelope plasmid encodes the G envelope glycoprotein of vesicular stomatitis virus (VSV G) envelope. VSV G envelope pseudotypes the lentivirus vector particles, producing vectors with broad tropism, high infectivity titers, and greater stability over other envelope pseudotypes.
3. The HIV transfer vector encodes the gene or cDNA of interest. The transfer vector retains the *cis*-acting elements of HIV required for packaging the vector RNA into the vector, reverse transcription, integration, and transcription: the LTR, packaging signal, primer binding site, and polyadenylation signal.

The 2nd generation lentivirus vectors were designed without coding regions for any accessory sequences in the packaging plasmid. Deletion of accessory genes seems to have no effect on vector production and transduction of dividing and many types of non-dividing cells, while increasing the safety margin by reducing the chance of generating RCL. However, some accessory-gene deleted HIV vectors are less efficient in transducing certain cell types, such as resting lymphocytes (6, 8), when compared with vectors that retain accessory genes.

3rd generation HIV vectors incorporated two additional safety features. First, is the use of self-inactivating (SIN) vectors. SIN vectors have a deletion in the enhancer region of the 3' U3 of the long terminal repeat (LTR). The 3' U3 deletion is transferred to the 5' LTR during the process of reverse transcription. The result is generation of a transcriptionally inactive vector that can not be converted into a full-length RNA, thus reducing the likelihood of RCR generation. The use of gammaretroviral SINs were hampered due to reduction of titer caused by the U3 deletion (35). However, use of U3-deleted HIV SIN vectors results in similar production titers and transgene expression to those of 1st or 2nd generation HIV vectors (37). Thus, SIN may be more efficient for adaptation to lentivirus vectors than to gammaretroviral (MLV) vectors. Additionally, the use of HIV SIN vectors hampers mobilization by wild-type HIV in infected cells (37). A second modification found in some 3rd generation vectors is the removal of the *tat* gene from the packaging vector, retaining only 3 of the original 9 HIV genes. The Tat protein has been shown to be dispensable when the 5' LTR is replaced with a heterologous constitutive promoter (20). The incorporation of safety features in 3rd generation HIV vectors is extensive and appears to have minimal effect on vector titer.

Other lentivirus vector developments include the following:

1. Splitting the packaging vector by :
    a) Expressing *rev* on a separate expression plasmid (9); therefore, the helper sequences are distributed on three different plasmids. This would require three recombination events to generate an RCL.

b) Additional separation of the *gag-pol* coding region onto two plasmids (34):

- Gag, protease, vif, tat, rev, and the Rev-response element (RRE) are encoded on one plasmid;
- Vpr, reverse transcriptase, integrase and the Rev-response element (RRE) are encoded on a second plasmid.

2. Development of stable packaging cell lines based on 3rd generation technology (15).
3. Development of non-HIV lentiviral vectors based upon EIAV, SIV, or FIV (17, 19, 29)

D. Detection of recombinants and replication-competent lentivirus (RCL)

In addition to incorporating elements into vector design that minimize vector recombination and generation of RCL, it is also essential to have sensitive assays for detection of RCL. A number of assays have been developed and evaluated for detection of recombination intermediates or for detection of RCL:

1. Detection of RCL by infectivity assay

A standard assay, similar to that used to detect RCR in gammaretroviral vectors, involves amplification of contaminating replicating virus by several passages of vector-containing supernatant or vector-producing cells on a permissive cell line coupled to a sensitive detection assay at the culture endpoint. For example, detection of amplified RCL may be achieved by an endpoint assay for HIV p24. An alternative detection assay would be used if the initial amplification cells carry a lentivirus vector with a marker gene. Supernatant of the amplified cells can then be used to infect naïve permissive target cells, whereby the detection of the mobilized expressed marker gene (for example, β-galactosidase expression) would indicate presence of replicating virus in the initial test article. (This approach is sometimes called vector rescue assay.) The choice of a positive control may be problematic, since the generation of a replication-competent VSV G-pseudotyped lentivirus may not be desirable. However, use of wild-type HIV as a positive control may not accurately reflect the sensitivity of detection of the predicted RCL from a vector production system, as the vector-derived RCL may differ significantly in its genetic structure from wild-type HIV.

2. Testing for helper sequences by functional assay

a) Transfer of the *tat* gene. This assay tests for recombinants that can express functional Tat protein. The assay relies on Tat-transactivation of an LTR-reporter gene construct in the target cell. In the absence of Tat, no LTR-driven expression is seen (24).

b) Test for recombination intermediates. This system has the advantage of being able to detect a recombinant that is one event away from becoming replication competent (34). For example, if a LTR-*gag-pol*-LTR

recombinant were packaged in the vector particles, there would be no replication in a naïve cell. If the *env* gene is supplied in *trans* in the target cell, potentially replication-competent virus could now arise by pseudotyping or by recombination. Additionally, if the patient population is HIV^+, one might want to test for recombinants that are not replication competent but can recombine with HIV sequences in the patient's own cells. If the HIV *env* gene was present in the target cell, the vector could recombine and become replication competent. Therefore, this assay may have added utility when the patient population is HIV^+.

3. Detection of helper sequences by PCR

One could test directly for presence of helper sequences in the vector final supernatant by PCR assay. While this could potentially be the most sensitive and rapid assay available, it is not the most biologically relevant and is prone to false positive results.

II. Preclinical Models for Safety Assessment of Lentivirus Vectors

Several biosafety issues need to be considered prior to the clinical application of lentiviral vectors, such as generation of replication-competent virus, recombination with wild-type HIV or other retroviridae, or mobilization of vector from transduced to non-target cells. These issues highlight the need to develop sensitive preclinical models to assess the potential risks of recombination, mobilization, and infection of non-target tissues in a suitable *in vivo* animal model.

A. Current recommendations for preclinical testing of gene transfer vectors

CBER's current recommendations to sponsors conducting gene transfer clinical trials are to consider the intended clinical use of the vector, any known toxicities associated with the class of vector under investigation, and any toxicities related to expression of the transgene. The preclinical toxicology program should then be designed to address each of these concerns (28). This individualized approach allows safety data to be generated that incorporate not only the specific concerns regarding the patient population and the transgene product, but also more general information about the toxicities associated with a particular vector class. Sponsors are also encouraged to incorporate data from other published studies using the same class of vector as supporting evidence for the safety evaluation of their product and to publish their own findings as a means of advancing the understanding of the toxicities of these agents.

CBER's published guidance document provides a framework for the design of preclinical safety programs in gene therapy, based on the available data from both *in vitro* and *in vivo* efficacy models, as well any specific concerns for the clinical population planned for study (1). The CBER document follows the general guidance set forth by the International Congress on Harmonisation S6 document, "Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals" (ICH S6) (2). Although the ICH guidance does not directly address toxicology study design for gene transfer agents, CBER recommends

that toxicity study design for gene transfer agents follow many of the principles set forth by ICH S6 regarding dose and species selection, route of administration, and study timing.

B. Selection of species for preclinical toxicology testing

CBER's recommendations for selection of species for safety evaluation have generally followed the guidance set forth by the ICH S6 document, taking into account the limitations of the animal model being tested. In summary, safety evaluation and toxicology testing in a single, relevant species, with sufficient, scientific justification provided for the use of that species is permissible to support initial entry into a phase 1 clinical trial. A relevant species can be determined by consideration for the clinical population and/or intended route of administration or by the species-specificity limitations of the transgene product or the gene transfer vectors. In some cases, the interaction of the transgene product with its specific receptor occurs only in humans and non-human primates, necessitating toxicology testing in monkeys. For many other gene transfer protocols, however, the toxicities observed are independent of the transgene product (*e.g.*, inflammatory reactions in response to adenovirus capsid proteins) and may be tested in rodents or other small, non-rodent laboratory species. In yet other cases, specific information regarding the safety of a gene transfer approach may only be obtained in an animal model of the disease in which the underlying disease pathology may contribute significantly to the safety or toxicity of the intervention.

A policy conference sponsored by the NIH Office of Biotechnology Activities in conjunction with the Recombinant DNA Advisory Committee and FDA/CBER was held in March, 1998. The general consensus from that meeting was that the issues involved in designing and developing appropriate preclinical testing to determine the safety of recombinant lentiviruses as vectors for gene transfer will be similar to those encountered for other viral or plasmid DNA vectors (3). However, the potential for vector recombination, trafficking to non-target tissues after *in vivo* administration, and interactions of the host immune system with the transduced cell were identified as areas requiring additional, targeted preclinical studies to adequately address the safety of these vectors prior to initial use in human trials. During the ensuing discussion, the consensus was that toxicology testing programs for novel lentivirus vectors should identify safety concerns anticipated for the clinical trial based on the intended use (*e.g., ex vivo* transduced cells or *in vivo* direct administration of vector) and on the duration of gene expression from these vectors. The recommendation from the conference was that further *in vivo* safety and toxicity testing to address these specific concerns should be performed with the vector on a "case-by-case" basis in an animal species relevant to the clinical model (3). In some cases, preclinical efficacy and bioactivity data for lentiviral vectors have been obtained in rat or mouse models of retinal degenerative diseases, hemophilia, or β-glucuronidase deficiency (5) (22) (27). In others, rhesus macaques have been used to study efficiency of lentiviral-mediated gene transfer and duration of gene expression in chemically-induced models of Parkinson's disease (16). There was no consensus on the choice of any one species over another as preferred for toxicology testing; rather, it was proposed that, where feasible, safety data should be collected during preclinical pharmacology and bioactivity testing in the animal model of the disease.

III. Special Considerations for Clinical Development of Lentivirus Vectors

The safety issues associated with the clinical use of lentiviral vectors overlap, in part, with those issues of concern with retroviral vectors based on gammaretroviruses: the potential for development of replication-competent virus and the long-term potential for tumorigenicity from integration into the chromosome. These issues are presently addressed by sensitive assays for RCR on vector production materials coupled with long-term follow-up of patients treated with gammaretroviruses, including screening of study subjects for evidence of infection by RCR and annual physical examinations. Safety concerns with lentivirus vectors will likely vary depending upon the specifics of the clinical trial, such as the patient population, clinical indication, route of administration (*ex vivo* vs. in vivo), and clinical endpoints.

On October 26, 2001, the BRMAC will hear about and consider one specific clinical trial being proposed by VIRxSYS Corporation for treatment of HIV-positive subjects. Two additional safety concerns regarding lentiviral vector safety have been identified if the subjects in the clinical trial are HIV-positive.

1. There exists the potential for recombination events between the lentiviral vector and wild-type HIV strain(s) in subjects who are HIV-positive. The consequences of such recombination events could be neutral, could reduce the replication rate or pathogenicity of the subject's virus, or could increase the replication rate or pathogenicity of the subject's virus. Unfortunately, with the natural variation of HIV strains, it is not possible to predict the outcome of such events. Since the development of a strain with increased pathogenicity would pose greater risk to both the patient and their close contact(s), it seems reasonable, therefore, to consider monitoring for recombination between the lentiviral vector and wild-type HIV strains present in subjects that are HIV-positive.

2. Lentiviral vectors carry the additional risks of mobilization by wild-type HIV. If the vector is designed to prevent or reduce HIV replication, some have argued that mobilization could enhance the therapeutic benefit (10). However, the true risks associated with mobilization are unknown. Therefore, consideration should be given to developing methods to assess mobilization of vector sequences to additional cells in clinical trial subjects.

In conclusion, while HIV-based vectors hold much promise for successful gene transfer, the known human pathogenicity of wild-type HIV has justifiably placed safety as a driving issue in lentiviral vector development.

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DRAFT Questions for October 25:
General Questions on Lentivirus Vectors

1. What safety data should be available prior to initial use of HIV-based lentivirus vectors in phase 1 clinical trials? Please consider the following:
    a) Replication-competent lentivirus (RCL)
    b) Recombination between vector and wild-type HIV
    c) Mobilization of vector by wild-type HIV

2. What should be the appropriate species for *in vivo*, preclinical safety and toxicology evaluation of lentivirus vectors? Please consider the following:
    a) Wild-type HIV-1 does not infect monocytes, lymphocytes, or other target cells in rodents nor in cynomologous or rhesus macaques and will only poorly infect $CD4^+$ T lymphocytes from chimpanzees, so mobilization studies will be complicated
    b) Lentivirus vectors pseudotyped with different envelopes (i.e. VSV-G, rabies envelope, flaviviruses) may have expanded cell tropisms, but the infection may be limited (for example, mouse cells have multiple blocks to HIV replication in addition to receptor-mediated).

3. Given the limitations of the available animal models for study of vector safety and mobilization, please comment on whether in vitro assays are sufficient to address the safety issues of recombination, RCL generation, and rescue and/or mobilization of lentiviral vectors, assuming such assays were accompanied by limited safety data from *in vivo* preclinical proof-of-concept studies?

DRAFT QUESTIONS FOR OCTOBER 26:
Questions Specific to VIRxSYS' Proposed Clinical Trial

1. Is the VRX496 vector proposed for use in the clinical trial by VIRxSYS designed and manufactured in a manner to sufficiently address safety concerns relevant to generation of RCL? Please consider that the vector will be used in HIV-positive subjects.

   How does the use of a transient transfection system vs. a stable packaging cell line for vector production affect the rate of recombination in a manner that would sufficiently compensate for the use of one plasmid to encode all helper functions?

2. Please discuss whether any additional safety testing of VRX496 should be performed prior to initiating the proposed clinical trial. In particular, please discuss the following:
   a) Should an in vitro assay for detection of functional LTR-*gag*-*pol*-LTR recombination intermediates be used as a lot release assay?
   b) Is the RCL infectivity assay of sufficient sensitivity? Is the positive control for the assay adequate for determining the sensitivity?
   c) Are there additional in vivo studies that need to be performed?
   d) When VRX496-transduced cells are challenged with wild-type HIV, a "breakthrough" virus is observed to replicate to high titers after a lag of 2-3 weeks. Is it necessary to characterize the molecular nature of the "breakthrough" virus prior to starting a clinical trial?

3. Please discuss whether vector mobilization is considered an advantage or a safety concern for the proposed clinical trial? Please consider the following:
   a) Are the data available from the assays to assess vector mobilization by wild-type HIV sufficient? Are there additional preclinical studies to assess vector mobilization that should be performed? If so, please discuss the optimal study design.
   b) Should assays for assessment of vector mobilization in the study subjects be developed? If so, please discuss the optimal assay design.

4. Please discuss whether there are any additional assays that should be used for safety assessment of the subjects in this clinical trial. In particular, should VIRxSYS monitor HIV variants present in the subject prior to and after treatment?